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ORIGINAL ARTICLE

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Micromolar taxol, with or without hyperthermia, induces mitotic catastrophe and cell necrosis in HeLa cells

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Abstract *Purpose*: Although the mode of action of taxol, when used in nanomolar or micromolar concentrations during long periods, is extensively studied, there are few data available on taxol-mediated cytotoxicity when the drug is applied for a short time alone or in combination with hyperthermia. We studied the effect of taxol and hyperthermia on cell cycle kinetics, proliferation, and mode of cell death in human cervical carcinoma HeLa cells, following a scheme which resembles the one currently used in regional chemotherapy. *Methods*: Cells were incubated with micromolar doses of taxol for two h under normothermic or hyperthermic conditions and then cultured in drug-free medium for several days. Cell viability was assessed via an MTT assay. Necrotic and apoptotic cell death was determined using Trypan blue staining and TUNNEL assay, respectively. Flow cytometry was used for the analysis of cell cycle kinetics and the counting of apoptotic cells. Mitotic index, nuclear morphology and nuclear envelope organization were analyzed by fluorescence microscopy. *Results*: Cells exposed to micromolar doses of taxol for 2 h and then transferred to a drug-free medium for 24 h were arrested at G2/M or M phase. When treated cells were cultured in normal media for longer periods, most of them remained in a tetraploid state, became multinucleated without properly completing cytokinesis and died mostly by necrosis. Hyperthermia alone exerted a cytotoxic effect, inhibited proliferation and caused minor changes in cell cycle kinetics. When combined with taxol treatment, hyperthermia modified the cell cycle-arresting effects of the drug, but did not alter significantly taxol-mediated cytotoxicity. *Conclusions*: From these data we conclude that short time incubation of HeLa cells under normothermic or hyperthermic conditions with micromolar concentrations of taxol is sufficient enough to induce extended cell growth arrest and cell death by necrosis.

Keywords Taxol · Hyperthermia · Cell necrosis · Cell cycle

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Introduction

Taxol (paclitaxel) is a plant alkaloid commonly used in the treatment of human carcinomas [24]. It binds with high affinity to the β-subunit of tubulin, causing decreased dynamic instability and increased microtubule rigidity [2, 19]. The cellular effects of the drug vary, depending on dose and treatment scheme. When applied for 20–24 h in the nanomolar range, it induces sustained mitotic arrest [14], inhibits protein prenylation [9], affects nuclear envelope organization, alters macromolecular trafficking through the nuclear pore complex [27] and triggers apoptosis [15, 27, 29]. At micromolar doses, in addition to mitotic arrest, taxol exerts other effects, which occur almost immediately: it promotes synthesis and release of cytokines [4, 12, 18] and induces "early response" genes, including those that encode tumor suppressors [21]. Mitotic blockade may not always culminate in apoptosis, since MCF-7 cells treated with various concentrations of taxol are partly blocked at G2/M phase, but do not become apoptotic [30]. Moreover, treatment of other breast cell lines with taxol could result either in apoptosis, or in necrosis, depending on dose [30].

Taxol-treated cells have been observed to develop multiple or lobulated nuclei. For instance, human endometrial carcinoma cells (Ishikawa) treated with low concentrations of the drug and allowed to complete mitosis, re-enter the cell cycle, effectively complete mitosis and cross the G1 checkpoint [27]. Roughly half of these cells have atypical nuclear shapes, with abnormally arranged nuclear pores and numerous gaps in the nuclear lamina meshwork. The lobulated and multinucleated cells remain alive for at least 24 h, undergoing apoptosis 72 h after removal of the drug [27] Alternatively, cells that fail to complete cytokinesis, after mitotic arrest caused by DNA or spindle damage, become multinucleated and undergo a non-apoptotic form of cell death known as mitotic catastrophe [5, 13].

The combination of different treatment modalities could be a significant factor in controlling primary tumor and metastatic disease. For instance, local hyperthermia enhances the cytotoxicity of some chemotherapeutic agents ([20] and references therein). The impact of hyperthermia on the cytotoxicity of taxol is currently debated.

In three in vivo studies, regional hyperthermia (1 h, 43°C) has been shown to enhance the effectiveness of paclitaxel against mouse adenocarcinoma [7, 8] and murine melanoma, [26], while in another report, hyperthermia (30 min, 41.5°C) did not improve paclitaxel's cytotoxicity against murine fibrosarcoma [20].

In vitro results are also contradictory and seem to depend on cell line and treatment protocol. When hyperthermia at 41°C was applied during the last hour of a 24 h exposure to 100 nM taxol, it did not enhance the cytotoxicity of the drug in either wild type or p53-inactivated human colorectal cell lines [28]. Similarly, when MCF-7 cells were heated (43°C, 1 h) at the beginning or in the middle of a 24-h taxol treatment, hyperthermia protected against the toxic effects of taxol at all concentrations tested (5, 10 and 100 nM). In addition, hyperthermic treatment has been reported to inhibit taxol-related cell cycle effects and cytotoxicity, despite the fact that the heated cells contained higher concentrations of the drug [17]. Finally, no thermal enhancement of cytotoxicity for paclitaxel and docetaxel has been observed in R1, SW 1573 and L-929 cells [23]. Contrasting these results, a synergistic and anti-proliferative effect has been reported after treatment of FM3A murine breast cancer cells with high concentration (10 μM) of paclitaxel for 1 h followed by exposure to 43°C for 1 h [22]. Moreover, brief hyperthermia (39.5°C, 90 min) on non-small cell lung, melanoma and fibrosarcoma cell lines and subsequent culture of treated cells under normal culture conditions for 3 days showed significant enhancement of paclitaxel-mediated toxicity, which consisted on the decrease of the paclitaxel IC50 [25].

From these observations it appears that the combined effect of taxol treatment and hyperthermia is determined by three important parameters: taxol concentration, duration of treatment and extent of heating. In this study, we have employed HeLa cells and investigated the cytotoxic effect of high concentrations of taxol, alone or in combination with hyperthermia. Observations presented below reveal: (1) a cytotoxic effect of taxol, which is mainly attributed to cell necrosis; and (2) a non-additive/non-inhibitory effect of hyperthermia (41.5°C) on taxol-mediated cytotoxicity.

Material and methods

Cell lines and culture

Cell line from cervical adenocarcinoma (HeLa), obtained from American Type Tissue Culture Collection (Manassas, VA, USA), was maintained at 37°C in a humidified atmosphere containing 5% CO_2. HeLa cells were cultured in Dulbecco's MEM (Biochrom, Berlin, Germany), supplemented with 10% heat-inactivated fetal bovine serum, penicillin and streptomycin.

Cell treatments

Upon reaching 50–60% confluence, cells grown either on culture dishes or on coverslips were treated with Taxol (Bristol-Meyer Squibb Company) 5, 10 or 20 μM prepared in culture medium, at 37, 41.5 or 43°C for 2 h, washed with fresh medium and incubated in a drug free medium at 37°C for up to 7 days. Hyperthermic treatment was performed by washing and incubating the cells with medium at the appropriate temperature and/or placing culture dishes in a CO_2 incubator regulated at 41.5 or 43°C.

Cell proliferation and cell death assays

Cells were grown on 24-well plates and after the various treatments proliferation was determined using MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Thiazol blue), as specified by the supplier (Sigma Chemical Co, St Louis, MO, USA). The percentages of viable cells for each treatment were determined by measurement of MTT absorbance, relative to the final cell population in non-treated cultures. All cell proliferation data shown are the means of eight measurements from three independent experiments.

The number of alive and dead cells was determined by incubating a fraction of the total cells (adherent and detached) with Trypan blue for 5 min, placing the cell suspension in a Neubauer hemocytometer and counting the number of figures that either incorporated (dead), or excluded (alive) the dye.

Indirect Immunofluorescence Microscopy

Indirect immunofluorescence was performed as described previously [27]. Briefly, cells grown on coverslips

were washed with PBS, fixed in 4% formaldehyde for 10 min at room temperature and permeabilized with Triton X-100. The antibodies used included: an anti-α-tubulin monoclonal (clone DM1A) obtained from Sigma Chemical Co; an anti-nucleoporin p68 autoimmune serum; an anti-lamin B polyclonal and an anti-emerin polyclonal ([27] and references therein). The specimens were visualized in a Leica SP confocal microscope.

The mitotic index and the morphology of interphase nuclei were assessed using total cells (adherent and in suspension), after staining with the DNA-binding dye, DAPI. At least 1,000 nuclei per sample were scored using a conventional fluorescence microscope, as mitotic (cells in prophase, metaphase, anaphase or telophase), normal-shaped or multinucleated.

Cell cycle analysis

Adherent cells were trypsinized, washed with PBS and treated in order to stain the DNA by the DNA-Prep Coulter Reagent Kit (Beckman Coulter). Samples were subjected to flow cytometry (FACS) in a Beckman Coulter Epics Elite.

Detection of Apoptosis

Apoptosis was assayed by TdT-mediated dUTP nick-end Labeling (TUNEL) assay. TUNEL assays were performed using an in situ cell death detection kit obtained from Boehringer Mannheim, according to the manufacturer's instructions. All samples were analyzed by flow cytometry in a Coulter Epics Elite.

Results

Inhibition of cell growth

After treatment with 10 or 20 μM taxol at 37, 41.5 or 43°C for 2 h and subsequent culture in drug-free medium for 3 days, cells exhibited retarded proliferation. To differentiate between cytostatic and cytotoxic effects, we compared cell viability at the end of the treatment with the number of viable cells before any treatment (initial population; Fig 1, white bars). Percentages of viable cells above the initial population denoted a cytostatic effect, whereas those below showed cytotoxicity. As shown in Fig. 1a, hyperthermia at 41.5°C exerted a cytostatic effect, whereas a raising the temperature to 43°C was cytotoxic. No substantial variations in cell proliferation were noted when taxol was applied alone or in combination with hyperthermia at 41.5 or 43°C.

When cells treated as described above were cultured in drug-free medium for longer periods (7 days, Fig. 1b), we showed that hyperthermia alone had no longer an anti-proliferating action, whereas no viable cells were detected after treatment with taxol combined or not with hyperthermia.

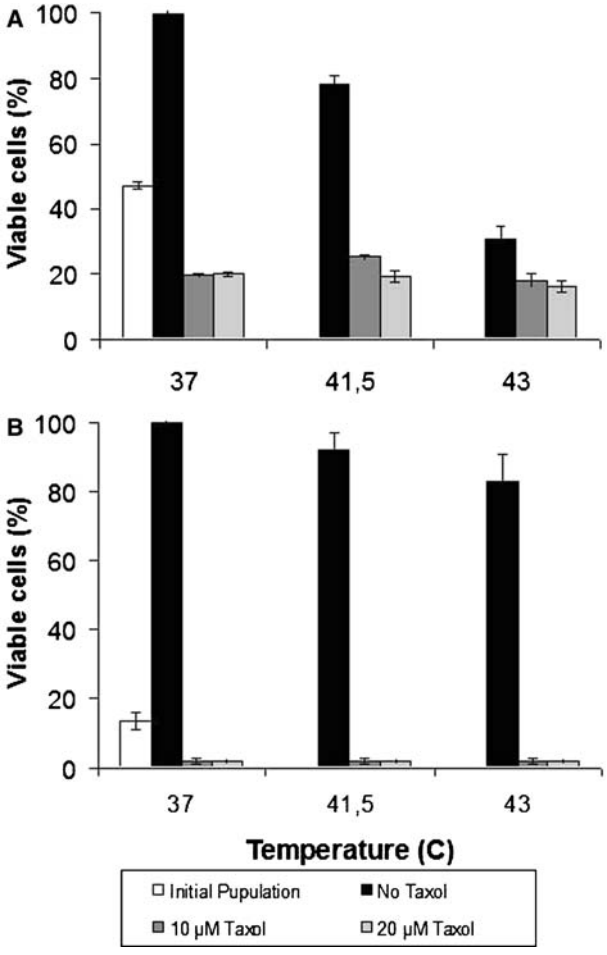

Fig. 1 Effect of taxol and hyperthermia on cell proliferation. An initial population of HeLa cells (*open bars*) was treated with 0, 10 or 20 μM of taxol for 2 h at 37, 41.5 or 43°C and then cultured in drug free medium at 37°C for 3 (**a**) or 7 (**b**) days. Cell proliferation was estimated using the MTT assay, as described in Materials and Methods. Results are expressed as percentages of viable cells by comparison to the maximal (100%) cell proliferation of normal cultures (no taxol, 37°C). Values are means ± SE of eight measurements in three separate experiments

Effects on cell cycle progression

To examine the parameters that contribute to the anti-proliferative effects, we studied cell cycle kinetics by FACS analysis. Cells were first treated either with hyperthermia (41.5°C, 2 h), or 10 μM taxol (with or without hyperthermia), and then cultured at 37°C in the absence of drug for 1–3 days. Hyperthermia, when applied alone, induces a slight disturbance on cell cycle kinetics. More specifically, immediately after treatment (Fig. 2, point "0") we noticed a decrease in the S sub-population, with a parallel increase of G2/M cells (Fig. 2, panel *Hyperthermia*). This was a transient effect, as 2 days after treatment, the percentage of G2/M phase

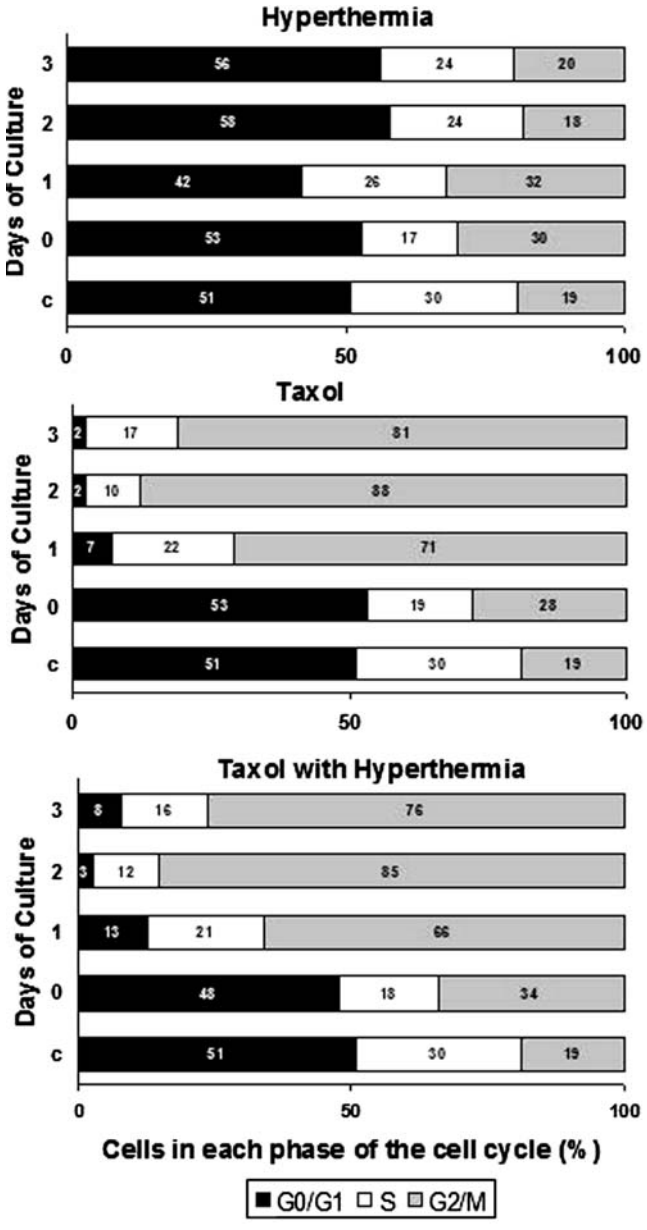

Fig. 2 Cell cycle analysis. The distribution of cells in the different phases of the cell cycle was estimated in cultures growing under normal conditions (c) and in cultures treated for 2 h with hyperthermia at 41.5°C (Hyperthermia), taxol 10 μM (Taxol) and both taxol and hyperthermia (Taxol with Hyperthermia). Cell cycle analysis was performed immediately after treatment (0), and 1, 2 and 3 days after cells been cultured under normal conditions. Total (adherent and non-adherent) cells were collected, stained with propidium iodide and analysed by flow cytometry as described in Materials and Methods. Results are expressed as the percentage of each sub-population and are representative of three independent experiments

became similar to those in controls. More than 70% of the cells treated with taxol alone remained arrested in the G2/M phase of the cell cycle for at least three days after release from the drug, indicating a sustained accumulation of mitotic or interphase tetraploid cells (Fig. 2, panel *Taxol*). When taxol was combined with hyperthermia (Fig. 2, panel *Taxol with Hyperthermia*), the percentages of the G2/M subpopulation were similar to those observed for cells treated with taxol alone (compare panels *Taxol* and *Taxol with Hyperthermia*).

Cell cycle sub-staging

Since the G2/M subpopulation is actually a mixture of interphase (G2) and mitotic (M) cells, to distinguish between these two phases, we counted mitotic figures after DAPI staining. Examination of more than 1,000 cells for each treatment protocol revealed that the number of mitotic cells differed significantly among the treatments (Fig. 3). The G2/M sub-population of cells treated with taxol and then cultured for one day in drug-free medium was composed exclusively of mitotic cells, whereas the corresponding population of cells treated with taxol and hyperthermia was a mixture of approximately 30% interphase G2 cells with normal looking nuclei and 70% mitotic cells (compare Fig. 2 and 3). When cells treated with taxol were cultured in drug free medium for one more day, we showed a direct (proportional) relationship between the number of mitotic and multinucleated cells (Fig. 3, *Taxol 37*C). In contrast, when hyperthermia at 41.5°C was combined with taxol treatment, the percentage of mitotic cells increased slightly the second day of culture and then decreased constantly until the fifth day, where less than 5% of mitotic cells were counted (Fig. 3, *Taxol 41.5C*). Multinucleated cells were observed the second day of culture and reached almost a plateau the third day. Our results show delayed accumulation of multinucleated cells when cells were treated with taxol plus hyperthermia instead of taxol alone. Multinucleated cells in both treatments presented nuclear envelope aberrations as described earlier [27]. As shown in Fig. 3a, b there are numerous deficits in the nuclear lamina/inner nuclear membrane and clustering of the nuclear pores.

By inspecting a large number of HeLa cells treated with taxol at 37°C or 41.5°C, we observed that almost all mitotic cells were in metaphase or a "metaphase like" state. No anaphase figures were detected, even after three days in culture (Table 1). Moreover, we noticed many cells with ball-like DNA condensation that resembled micronuclei, indicating that multinucleated cells are produced by direct decondensation of chromosome clusters after a long period of mitotic arrest (Fig. 4, *asterisks*). Similar events have been described for mitotic catastrophe, a process that involves multinucleation and is mechanistically different from the common forms of apoptosis [5, 13].

Mode of cell death

The results presented above indicated that the cytotoxicity of hyperthermia, taxol, or the combination of the

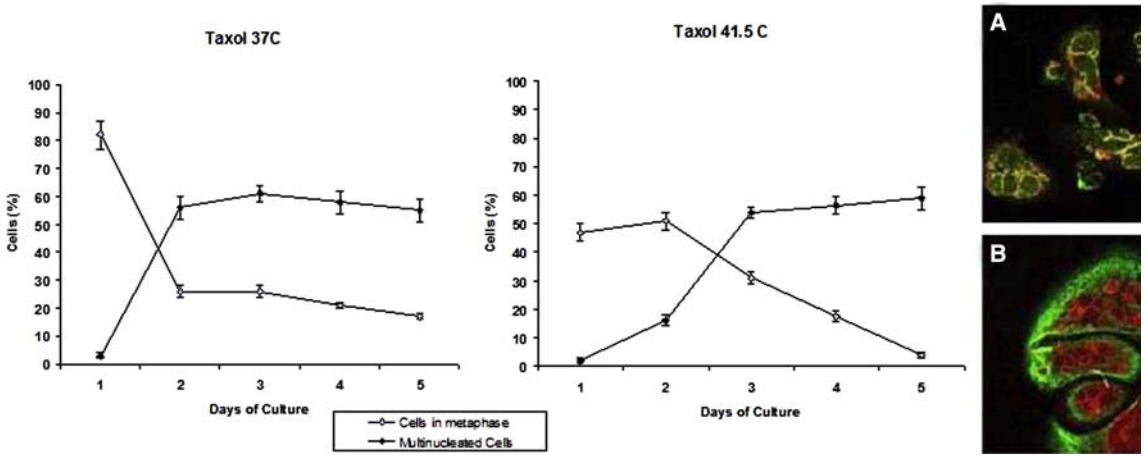

Fig. 3 Effect of taxol under normothermic and hyperthermic conditions on the mitotic arrest and the nuclear morphology of HeLa cells. Cells grown on 6-well plates were treated with taxol 10 μM for 2 hours at 37 or 41.5°C and then cultured under normal conditions for up to 5 days. Total (adherent and in suspension) HeLa cells were stained with DAPI and more than 1,000 specimens from each treatment were microscopically examined in order to determine the number of cells in metaphase (*open rhombs*) and cells with multinucleated morphology (*solid rhombs*). Results are expressed as a percentage of total cells and are representative of three independent experiments. Images (**a**) and (**b**) show nuclear envelope structural defects of cells grown on coverslips, treated with taxol 10 μM for two hours at 41.5°C and then cultured under normal conditions for three days. **a** Image shown the merge of cells stained with specific antibodies for nuclear pores (PBC68; *green*) and for inner nuclear membrane (emerin; *red*). Note the large gaps on the nuclear envelope integrity and the presence of nuclear pore clusters. **b** Image shown the merge of cells stained for microtubules (*green*) and the nuclear lamina (lamin **B**; *red*). Multinucleated abnormal cells derived from an aberrant cytokinesis are shown

two, is due to both inhibition of cell cycle progression and cell death.

To better define the relative contribution of necrosis and apoptosis in cell death, the total cell population (adherent and non-adherent cells) was analyzed using Trypan blue and DAPI staining, as well as TUNNEL assays. Cells were treated with 10 μM taxol at 37 or 41.5°C for 2 h and then cultured in normal media and temperature for 3 days. At 24-hr intervals, the cells were stained with Trypan blue, counted and the percentage of viable cells was determined. In parallel, apoptotic cells were quantified both by fluorescence microscopy and FACS analysis. Our results showed that hyperthermia had only a minor effect on both apoptotic and necrotic cell death (Fig. 5, panel *Hyperthermia*). Moreover, we observed that taxol's cytotoxicity is mainly due to necrosis. Approximately half the initial population died after two days in culture, whereas very few cells remained alive after three days. The percentage of apoptotic cells at that time did not exceed 18% of the total cell population, demonstrating that necrosis is the predominant cause of death (Fig.5, panel *Taxol*). Similar percentages on apoptotic and necrotic cells were measured when cells were treated with taxol plus hyperthermia (Fig.5, panel *Taxol with hyperthermia*).

Table 1 Distribution of mitotic cells in the different phases of mitosis [Cells grown on 6-well plates were incubated for 2 h at 41.5°C (Hyperthermia) or treated with taxol 10 μM for two hours at 37 (Taxol) or 41.5°C (Taxol plus Hyperthermia) and then cultured under normal conditions for 24, 48 and 72 h. Total (adherent and in suspension) HeLa cells were stained with DAPI and more than 1,000 specimens from each treatment were microscopically examined in order to determine the number of cells in the different phases of mitosis (prophase, metaphase/prometaphase, anaphase and telophase). Results are expressed as a percentage of mitotic cells and are representative of three independent experiments]

	Percentage of mitotic cells in			
	Prophase	Metaphase/ Prometaphase	Anaphase	Telophase
Hyperthermia (hours)				
24	16.7	55.5	16.7	11.1
48	6.2	81.2	6.3	6.3
72	2.1	66.8	14.5	16.6
Taxol (hours)				
24	0.4	99.6	0	0
48	0	100	0	0
72	0	100	0	0
Taxol plus Hyperthermia (hours)				
24	0.6	99.4	0	0
48	0	100	0	0
72	0	100	0	0

Discussion

The novel finding of this study is that a brief incubation of HeLa cells with micromolar concentrations of taxol has been found to induce cell death, as no proliferating cells are detected after 7 days of culture in drug-free medium. So far, in vitro studies on taxol-mediated cytotoxicity have been done using low (nanomolar concentrations) or high (micromolar concentrations) doses of the drug during long periods [3, 27, 30]. In this context, we have previously shown that treatment of

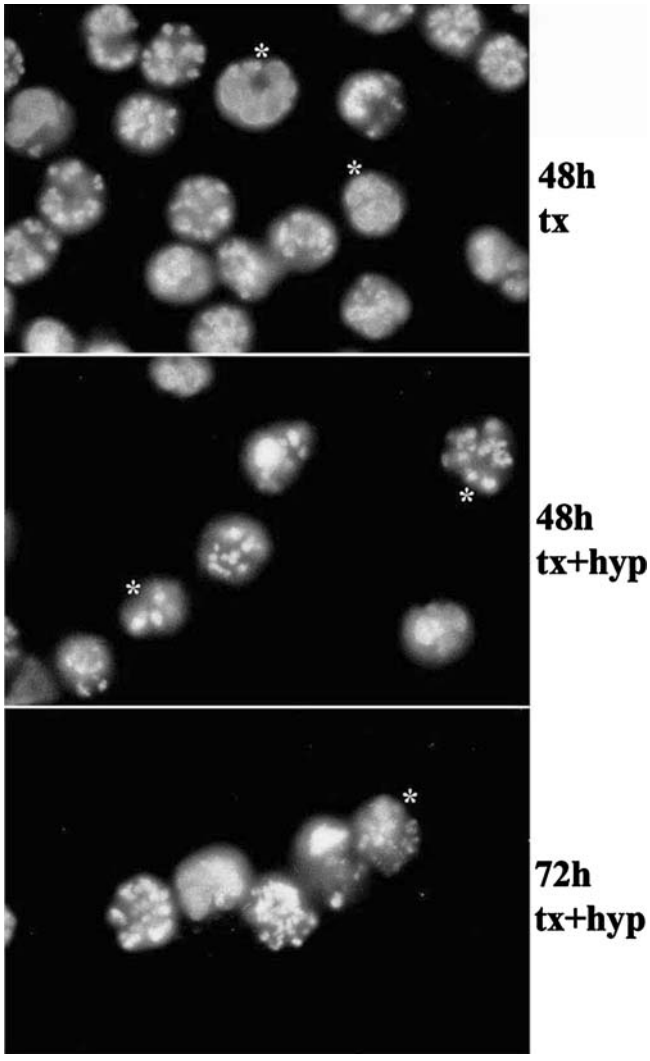

Fig. 4 Chromatin morphology of G2/M arrested HeLa cells. Cells grown on coverslips were treated with taxol 10 μM for 2 h at 37 (tx) or 41.5°C (tx + hyp) and then cultured under normal conditions for 48 or 72 h. Chromatin was stained with DAPI and specimens were examined under a fluorescent microscope

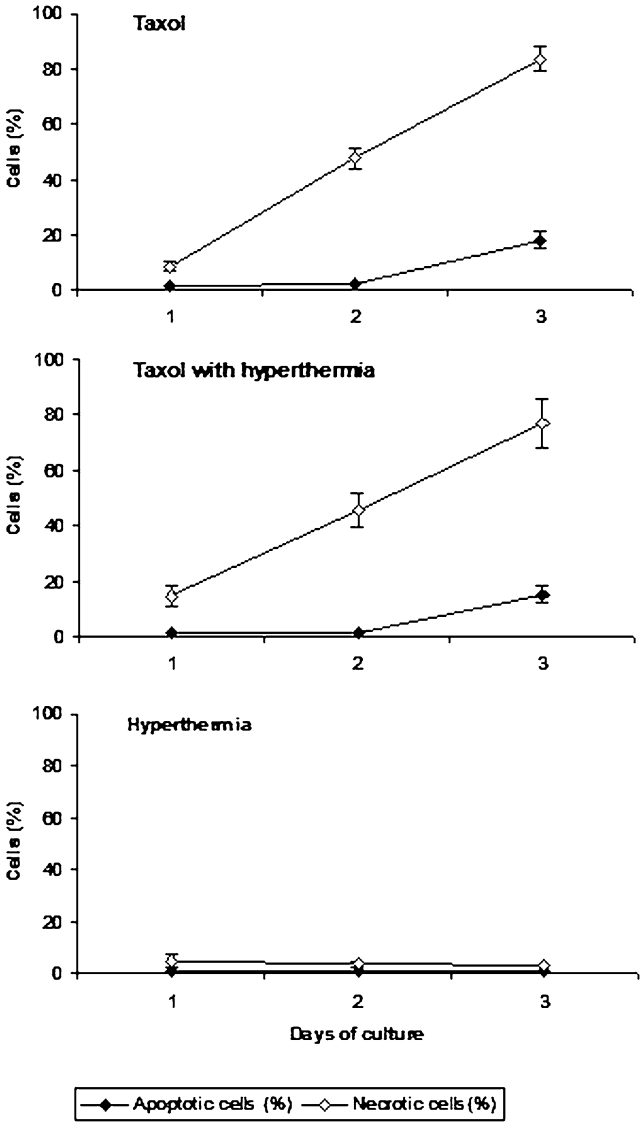

Fig. 5 Effect of taxol and hyperthermia on cell necrosis and apoptosis. HeLa cells were treated for 2 h with hyperthermia at 41.5°C or taxol 10 μM at 37 or 41.5°C and then cultured under normal conditions for up to three days. Total (adherent and no-adherent) cells were collected and stained either with Trypan blue for the determination of necrotic cells (*open rhombs*) or with TUNNEL for the measurement of apoptotic cells (*solid rhombs*) using flow cytometry as described in Materials and Methods. Results are expressed as a percentage of total cells and are representative of three independent experiments

Ishikawa cells for several hours with low concentrations of taxol is sufficient to inhibit cell proliferation and to block more than 80% of cells at the metaphase/anaphase transition. Cells released from the drug exited mitosis and reentered cell cycle, but presented structural and functional defects of the nuclear envelope, multinucleation and, finally, died by apoptosis [27].

In this study we show that HeLa cells treated for 2 h with micromolar concentrations are also efficiently arrested in the metaphase/anaphase transition. In contrast to that described for cells treated with low concentrations of taxol, upon release from the drug, the cells fail to undergo cytokinesis and progress through the cell cycle, as no anaphase or telophase figures have been observed. As a result, mitotic cells become multinucleated, probably by partial decondensation of mitotic chromosomes. These steps resemble mitotic catastrophe, an apoptosis-like process characterized by chromatin condensation and micronucleation but easily distinguishable from the common forms of apoptosis by the lack of DNA degradation [5, 13]. Thereafter, HeLa cells die very rapidly by necrosis. Although necrosis is not generally considered a common form of cell death after taxol treatment, there is at least one report which links cell necrosis at micromolar doses of taxol and apoptosis at nanomolar doses of the drug in various breast cancer cell lines [30]. In other cell lines such as A549 cells, no signal for apoptosis has been reported after treatment

with taxol concentrations that triggered G2/M arrest [6]. Moreover, Lane and Nigg [16] have reported that defects in a centrosome maturation checkpoint induce mitotic catastrophe in HeLa cells. The commonly observed phenotypes in such cells include rounded mitotic-like cells with condensed ball-like chromosomes and interphase cells with strikingly fragmented nuclei (micronuclei) or with multiple nuclei (multinucleated cells), phenotypes which resemble amazingly to those we have observed in this study. Finally, it has been reported [1] that non-transformed 3T3.A31 cells do not develop apoptosis, although they become characteristically micronucleated after treatment with a fluorescent taxoid (FLUTAX).

Several in vivo and in vitro studies concerning the treatment of cancer cells with taxol and hyperthermia have been published the last decade. The impact of hyperthermia on taxol-mediated cytotoxicity and cell cycle kinetics differs among these studies and seems to depend on cell line and the treatment scheme. In general, a lack of enhancement, or even a decrease, in the cytotoxicity due to taxol has been reported ([17, 28] and our unpublished results), as a consequence of the combination of 1 h hyperthermia at 41 or 43°C to long term exposure of various cell types with nanomolar concentrations of taxol. In this study we treated human cervical adenocarcinoma cells with micromolar concentrations of taxol and hyperthermia (41.5°C) for two hours, following a scheme which resembles the one currently used in regional, ie, intraperitoneal chemotherapy [10, 11]. In intraoperative hyperthermic intraperitoneal chemotherapy, the peritoneal cavity is perfused with high (micromolar) concentrations of taxanes for two hours. During the treatment, temperature is allowed to fluctuate between 41°C and 43°C and after completion, the fluid is dried from the abdominal cavity. In contrast, when the drug is administered via the systemic circulation, its concentration falls to the nanomolar range and persists for a long time.

Assessment of cell proliferation for up to seven days and measurement of cell necrosis/apoptosis showed that, although hyperthermia does not increase taxol-mediated cytotoxicity, the combined treatment of taxol and hyperthermia induces mitotic catastrophe, which leads to extensive necrosis. Our in vitro results have shown that hyperthermia had no long-term effects on the inhibition of cell proliferation. However, the cytostatic effect we have observed in the beginning of treatment may be of importance under in vivo conditions, where hyperthermia is expected to enhance vascular permeability of the tumor.

Apoptosis is the main cause of cell death when cancer cells are treated in vitro with paclitaxel for long periods. Such a treatment, when nanomolar concentrations of the drug are used, is clinically relevant in systemic chemotherapy. However, in addition to the apoptotic cell death, a desirable goal in cancer treatment is to increase local inflammation, which could result in induction of immunological activation. It is known that necrosis causes local inflammation that may be beneficial for the anti-tumor effect. In contrast, the process of apoptosis is non-inflammatory and therefore does not recruit the resources of the immune system. With this consideration, locoregional chemotherapy using the proposed scheme could be of clinical relevance and merits to be tested in appropriate preclinical models.

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References

1. Abal M, Souto AA, Amat-Guerri F, Acuna AU, Andreu JM, Barasoain I (2001) Centrosome and spindle pole microtubules are main targets of a fluorescent taxoid inducing cell death. Cell Motil Cytoskeleton 49:1–15
2. Arnal I, Wade RH (1995) How does taxol stabilize microtubules? Curr Biol 5:900–908
3. Blajeski AL, Kottke TJ, Kaufmann SH (2001) A multistep model for paclitaxel-induced apoptosis in human breast cancer cell lines. Exp Cell Res 270:277–288
4. Bogdan C, Ding A (1992) Taxol, a microtubule-stabilizing antineoplastic agent, induces expression of tumor necrosis factor alpha and interleukin-1 in macrophages. J Leukoc Biol 52:119–121
5. Chan TA, Hermeking H, Lengauer C, Kinzler KW, Vogelstein B (1999) 14–3-3Sigma is required to prevent mitotic catastrophe after DNA damage. Nature 401:616–620
6. Chen JG, Yang CP, Cammer M, Horwitz SB (2003) Gene expression and mitotic exit induced by microtubule-stabilizing drugs. Cancer Res 63:7891–7899
7. Cividalli A, Cruciani G, Livdi E, Pasqualetti P, Tirindelli Danesi D (1999) Hyperthermia enhances the response of paclitaxel and radiation in a mouse adenocarcinoma. Int J Radiat Oncol Biol Phys 44:407–412
8. Cividalli A, Livdi E, Ceciarelli F, Piscitelli M, Pasqualetti P, Cruciani G, Danesi DT (2000) Hyperthermia and paclitaxel–epirubicin chemotherapy: enhanced cytotoxic effect in a murine mammary adenocarcinoma. Int J Hyperthermia 16:61–71
9. Danesi R, Figg WD, Reed E, Myers CE (1995) Paclitaxel (taxol) inhibits protein isoprenylation and induces apoptosis in PC-3 human prostate cancer cells. Mol Pharmacol 47:1106–1111
10. de Bree E, Romanos J, Michalakis J, Relakis K, Georgoulias V, Melissas J, Tsiftsis DD (2003) Intraoperative hyperthermic intraperitoneal chemotherapy with docetaxel as second-line treatment for peritoneal carcinomatosis of gynaecological origin. Anticancer Res 23:3019–3027
11. de Bree E, Rosing H, Beijnen JH, Romanos J, Michalakis J, Georgoulias V, Tsiftsis DD (2003) Pharmacokinetic study of docetaxel in intraoperative hyperthermic i.p. chemotherapy for ovarian cancer. Anticancer Drugs 14:103–110
12. Ding AH, Porteu F, Sanchez E, Nathan CF (1990) Shared actions of endotoxin and taxol on TNF receptors and TNF release. Science 248:370–372
13. Erenpreisa JE, Ivanov A, Dekena G, Vitina A, Krampe R, Freivalds T, Selivanova G, Roach HI (2000) Arrest in metaphase and anatomy of mitotic catastrophe: mild heat shock in two human osteosarcoma cell lines. Cell Biol Int 24:61–70
14. Jordan MA, Toso RJ, Thrower D, Wilson L (1993) Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations. Proc Natl Acad Sci USA 90:9552–9556

15. Jordan MA, Wendell K, Gardiner S, Derry WB, Copp H, Wilson L (1996) Mitotic block induced in HeLa cells by low concentrations of paclitaxel (Taxol) results in abnormal mitotic exit and apoptotic cell death. Cancer Res 56:816–825
16. Lane HA, Nigg EA (1996) Antibody microinjection reveals an essential role for human polo-like kinase 1 (Plk1) in the functional maturation of mitotic centrosomes. J Cell Biol 135:1701–1713
17. Leal BZ, Meltz ML, Mohan N, Kuhn J, Prihoda TJ, Herman TS (1999) Interaction of hyperthermia with Taxol in human MCF-7 breast adenocarcinoma cells. Int J Hyperthermia 15:225–236
18. Lee LF, Schuerer-Maly CC, Lofquist AK, van Haaften-Day C, Ting JP, White CM, Martin BK, Haskill JS (1996) Taxol-dependent transcriptional activation of IL-8 expression in a subset of human ovarian cancer. Cancer Res 56:1303–1308
19. Mickey B, Howard J (1995) Rigidity of microtubules is increased by stabilizing agents. J Cell Biol 130:909–917
20. Mohamed F, Marchettini P, Stuart OA, Urano M, Sugarbaker PH (2003) Thermal enhancement of new chemotherapeutic agents at moderate hyperthermia. Ann Surg Oncol 10:463–468
21. Moos PJ, Fitzpatrick FA (1998) Taxane-mediated gene induction is independent of microtubule stabilization: induction of transcription regulators and enzymes that modulate inflammation and apoptosis. Proc Natl Acad Sci USA 95:3896–3901
22. Othman T, Goto S, Lee JB, Taimura A, Matsumoto T, Kosaka M (2001) Hyperthermic enhancement of the apoptotic and antiproliferative activities of paclitaxel. Pharmacology 62:208–212
23. Rietbroek RC, Katschinski DM, Reijers MH, Robins HI, Geerdink A, Tutsch K, d'Oleire F, Haveman J (1997) Lack of thermal enhancement for taxanes in vitro. Int J Hyperthermia 13:525–533
24. Rowinsky EK, Donehower RC (1995) Paclitaxel (taxol). N Engl J Med 332:1004–1014
25. Schrump DS, Zhai S, Nguyen DM, Weiser TS, Fisher BA, Terrill RE, Flynn BM, Duray PH, Figg WD (2002) Pharmacokinetics of paclitaxel administered by hyperthermic retrograde isolated lung perfusion techniques. J Thorac Cardiovasc Surg 123:686–694
26. Sharma D, Chelvi TP, Kaur J, Ralhan R (1998) Thermosensitive liposomal taxol formulation: heat-mediated targeted drug delivery in murine melanoma. Melanoma Res 8:240–244
27. Theodoropoulos PA, Polioudaki H, Kostaki O, Derdas SP, Georgoulias V, Dargemont C, Georgatos SD (1999) Taxol affects nuclear lamina and pore complex organization and inhibits import of karyophilic proteins into the cell nucleus. Cancer Res 59:4625–4633
28. van Bree C, Savonije JH, Franken NA, Haveman J, Bakker PJ (2000) The effect of p53-function on the sensitivity to paclitaxel with or without hyperthermia in human colorectal carcinoma cells. Int J Oncol 16:739–744
29. Woods CM, Zhu J, McQueney PA, Bollag D, Lazarides E (1995) Taxol-induced mitotic block triggers rapid onset of a p53-independent apoptotic pathway. Mol Med 1:506–526
30. Yeung TK, Germond C, Chen X, Wang Z (1999) The mode of action of taxol: apoptosis at low concentration and necrosis at high concentration. Biochem Biophys Res Commun 263:398–404